Short Communication: Fc Gamma Receptors IIa and IIIa Genetic Polymorphisms Do Not Predict HIV-1 Disease Progression in Kenyan Women

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Abstract

Genetic polymorphisms of the Fc gamma receptors (FcγR) IIa and IIIa have been implicated in the rate of HIV-1 disease progression, but results are inconsistent. We aimed to determine the association between these polymorphisms and disease progression in a cohort of HIV-1 seroconverters from Mombasa, Kenya. Neither FcγRIIa nor FcγRIIIa genotypes were predictive of set point viral load, viral load increase, CD4 decline, or HIV-1 disease progression (time to CD4 count <200 cells/mm³, death, or treatment initiation). Our results suggest that FcγR polymorphisms might not be an important indicator of viral control and disease progression in this population.

FCY RECEPTORS (FcγR) PRESENT on the surface of monocytes, macrophages, dendritic cells, and natural killer cells play a role in the immune response to HIV-1 infection by binding the Fc portion of immunoglobulin G (IgG). FcγRs including FcγRIIa (CD32) mediate phagocytosis of antibody-virion complexes, leading to degradation and clearance of the virus.¹ Additionally, FcγRIIIa (CD16) on natural killer cells can participate in antibody-dependent cellular cytotoxicity (ADCC) by binding to the Fc portion of an IgG whose Fab portion is bound to an infected cell expressing the HIV-1 envelope surface protein.^{1,2} Upon binding, natural killer cells release cytotoxic granules, killing the infected cell. In addition, binding leads to release of cytokines that contribute to antibody-dependent cell-mediated virus inhibition (ADCVI).³ Single nucleotide polymorphisms (SNPs) in the coding region of FcγRIIa and FcγRIIIa affect IgG binding affinity and thus can modulate these downstream functions.^{4,5}

FcγRIIa (CD32) is polymorphic at amino acid (AA) site 131, coding for either a histidine (H) or an arginine (R).⁴ A receptor with histidine at AA site 131 has higher affinity for all IgG subclasses than that with arginine.⁶ This is particularly pronounced with IgG2 and IgG3, where even one H allele present allows for higher affinity binding than an

RR homozygote.⁷ The difference in affinity for IgG has been linked to efficiency in phagocytosis of IgG-IHV complexes.^{8,9}

FcγRIIIa (CD16) is polymorphic at AA site 158 for either a valine (V) or phenylalanine (F). IgG1 and IgG3 bind with higher affinity to V-containing receptors than F-containing receptors.⁶ V receptors are also associated with higher levels of ADCC.¹⁰ In HIV-1 infection, increased ADCC has been associated with HIV-1 viral control and reduced ADCC has been associated with HIV-1 disease progression.^{11,12}

If alleles associated with higher binding affinity lead to better control of viremia, they should be associated with a slower rate of disease progression. Consistent with this hypothesis, Forthal *et al.* reported an association between the high-affinity FcγRIIa allele and slower progression in North American men.¹³ However, Poonia *et al.* reported that individuals with the high-affinity FcγRIIIa allele progressed more rapidly, raising the possibility that high-affinity alleles could enhance infection.¹⁴ To further explore these relationships we evaluated the impact of FcγR polymorphisms on HIV-1 disease progression using stored samples and data from a 20-year longitudinal seroconverter cohort of female sex workers from Mombasa, Kenya.

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This study included data from women who seroconverted while participating in a prospective cohort of initially HIV-1-seronegative female sex workers in Mombasa, Kenya, between 1993 and 2011.^{15–17} Blood was collected quarterly for CD4 counts and viral load measurements as described previously.^{18,19} Two hundred and fifty-three seroconverters with two or more viral loads and two or more CD4 counts recorded as well as samples available for genotyping were included in this study. The Mombasa Cohort was approved by the human subjects research committees of the Kenyatta National Hospital in Kenya and the University of Washington and Fred Hutchinson Cancer Research Center in Seattle. All participating women provided informed consent for research participation.

FcγRIIa and FcγRIIIa genotypes were obtained using TaqMan allelic discrimination assays with assay IDs C_9077561_20 and C_42463377_10, respectively (Life Technologies Carlsbad, CA).²⁰ Control peripheral blood mononuclear cells (PBMCs) of known genotype were obtained from Coriell Cell Repository (Camden, NJ). Control DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) and verified by Sanger sequencing. Seroconverter DNA was also isolated from lysed PBMCs. When PBMC DNA was not available, DNA was extracted from plasma samples using the same kit ($n=67$).

We analyzed the relationship between FcγR genotype and set point plasma viral load using linear regression, with genotype as a categorical predictor. Plasma viral load increase over time and CD4 count decline over time were analyzed with linear mixed effects (LME) models. The rate of disease progression was modeled with Cox proportional hazards, Kaplan–Meier curves, and log-rank tests. Primary predictors were FcγRIIa genotype (HH, HR, and RR) and FcγRIIIa genotype (FF, FV, or VV). Reference groups were selected based upon the low-risk groups previously reported: high-affinity FcγRIIa HH homozygotes and low-affinity FcγRIIIa FF homozygotes.^{13,14} Analyses were performed with STATA software (StataCorp LP, College Station, TX). Hardy–Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) tests were performed as quality control.

Set point viral load was considered the first viral load obtained between 4 and 24 months postinfection. Viral loads were log₁₀ transformed and CD4 cell counts were square root transformed to approximate normal distributions. Analyses were performed before and after adjustment for covariates associated with viral load. These were age at infection, use of hormonal contraception (depot medroxyprogesterone-acetate, oral contraceptive pills, Norplant) within a 70-day window of infection, and the presence of genital ulcer disease at infection.^{16,18} The LME analyses excluded viral load and CD4 measurements prior to set point. Women were censored after ART initiation. The final models for LME as well as linear regression included both FcγRIIa and FcγRIIIa genotypes, and an interaction between the genotypes was tested in the LME and Cox models. Cox proportional hazards regression was used to evaluate the association between FcγRIIa and FcγRIIIa genotype and time from infection to disease progression, defined as CD4 count <200 cells/mm³, ART initiation, or death.

Two hundred and fifty-three women were successfully genotyped for FcγRIIa, with distributions of 51 (20%) HH,

131 (52%) HR, and 71 (28%) RR. Two hundred and forty-nine women were successfully genotyped for FcγRIIIa, with distributions of 34 (13%) VV, 123 (49%) FV, and 92 (37%) FF. The sample population was in HWE for FcγRIIa ($\chi^2=0.49$, $p=0.50$) and FcγRIIIa ($\chi^2=0.50$, $p=0.48$). There was evidence of LD between FcγRIIa and FcγRIIIa (Pearson's χ^2 test for independence = 10.23, $p=0.04$). The frequency of these alleles was similar to those reported previously in many populations, including Kenyans.^{13,14,21–26}

The median age at seroconversion was 30 years (Table 1). The median cohort participation before seroconversion was 14.9 months and the median postinfection follow-up time was 69.0 months. Nineteen women had genital ulcer disease and 116 were using hormonal contraception at infection.

Two hundred and twenty of 253 (87.0%) women genotyped had viral load measured between 4 and 24 months postinfection. The mean log₁₀ set point viral load was 4.53 copies/ml (range, 2.00–7.22 copies/ml). Figure 1 compares mean set point. There was no difference in set point viral load for women with FcγRIIa HR [beta = +0.06, 95% confidence interval (CI) –0.25 to +0.36, $p=0.72$] or RR (beta = +0.07, 95% CI –0.27 to +0.40, $p=0.70$) relative to HH. Similarly, no difference was found between set point viral load for women with FcγRIIIa FV (beta = –0.17, 95% CI –0.45 to +0.11, $p=0.22$) and VV (beta = –0.21, 95% CI –0.56 to +0.14, $p=0.23$) compared to FF. These results did not change after adjustment for covariates.

Viral load incline over time was not associated with receptor genotype (Fig. 1). Compared to reference group FcγRIIa HH, neither the slope (HR beta = –0.0001, 95% CI –0.0002 to 0.0001, $p=0.22$; RR beta = –0.0001, 95% CI –0.0003 to 0.0006, $p=0.21$) nor the intercept differed (HR beta = 0.008, 95% CI –0.28 to 0.29, $p=0.95$, RR beta = –0.014, 95% CI –0.33 to 0.30, $p=0.93$), a result unchanged after adjustment. Compared to reference group FcγRIIIa FF, neither the slope (FV beta = –0.0008, 95% CI –0.0003 to 0.0002, $p=0.18$; VV beta = –0.0003, 95% CI –0.0002 to 0.0001, $p=0.71$) nor the intercept differed (FV beta = –0.18, 95% CI –0.42 to 0.062, $p=0.15$; VV beta = –0.10, 95% CI –0.45 to 0.24, $p=0.56$), which was also not changed by adjustment. Results were also unchanged after inclusion of an interaction term between genotypes.

TABLE 1. CHARACTERISTICS AND EXPOSURES OF 253 FEMALE KENYAN SEROCONVERTERS

<i>Study population characteristics</i>	<i>Median (IQR) or N (%)</i>
Age at seroconversion, years	30 (27–34)
Cohort participation before seroconversion, months	14.9 (5–32.1)
Postinfection follow-up time, months	69 (28.1–123.8)
Plasma viral load (log ₁₀ HIV-1 copies/ml)	4.8 (4.2–5.4)
CD4 ⁺ T cell count (cells/mm ³)	387 (262–552)
Square root of CD4 ⁺ T cell count (cells/mm ³)	19.7 (16.2–23.5)
Genital ulcer disease exposure at infection	19 (7.5%)
Hormonal contraception exposure at infection	116 (49.7%)

FIG. 1. Association between the Fc gamma receptors (FcγR) IIa and IIIa genetic polymorphisms and set point viral load, viral load over time, and CD4 cell decline over time. (A, B) The distribution of log₁₀ plasma viral load at viral set point, defined as the first viral load between 4 and 24 months postinfection, for women with FcγRIIa receptor genotypes HH, RH, and RR (A) and FcγRIIIa receptor genotypes FF, FV, and VV (B). (C, D) Linear mixed effects models (LME) of log₁₀ plasma viral load change over time. Change was modeled starting at 4 months postinfection, indicated by the gray dotted line and arrow in each panel. (C) FcγRIIa regression lines indicate the modeled intercept and slope (solid line = HH, dashed line = RH, and dotted line = RR). Raw data are represented by symbols (triangle = HH, circle = RH, square = RR). (D) FcγRIIIa regression lines indicate the modeled intercept and slope for FcγRIIIa by genotype (solid line = FF, dashed line = FV, and dotted line = VV). Raw data are represented by symbols (triangle = FF, circle = FV, square = VV). (E, F) LME models of square-root-transformed CD4 count change over time. Change was modeled starting at 4 months postinfection, indicated by the gray dotted line and arrow in each panel. (E) FcγRIIa regression lines and symbols are consistent with (C). (F) FcγRIIIa regression lines and symbols are consistent with (D).

Likewise, CD4 count decline over time was also not associated with receptor genotype (Fig. 1). Compared to reference group FcγRIIa HH, neither the slope (HR beta = 0.001, 95% CI -0.0006 to 0.002, $p = 0.063$; RR beta = 0.0007, 95% CI -0.0007 to 0.002, $p = 0.38$) nor the intercept differed (HR beta = -0.71, 95% CI -2.63 to 1.21, $p = 0.47$; RR beta = 1.47, 95% CI -0.70 to 3.64, $p = 0.18$), which did not change upon adjustment or inclusion of an interaction term

between genotypes. Compared to reference group FcγRIIIa FF, the FV slope (beta = -0.0008, 95% CI -0.0011 to 0.0009, $p = 0.87$) and intercept (beta = 0.50, 95% CI -1.12 to 2.12, $p = 0.55$) did not differ before or after adjustment. The FcγRIIIa VV slope was not different from FF (beta = -0.0003, 95% CI -0.002 to 0.001, $p = 0.87$). However, the VV intercept did differ from that of FF. This finding was of borderline significance in the unadjusted model (beta = 2.21,

FIG. 2. Association between Fc γ RIIa and Fc γ RIIIa genetic polymorphisms and HIV-1 disease progression. **(A)** Kaplan-Meier graph of Fc γ RIIa displaying time to event analysis for progression. Failure was defined as the time to the first of three failure events: CD4 count <200, ART initiation, or death. The survival curves represent the different receptors (*solid line* = HH, *dashed line* = RH, and *dotted line* = RR). **(B)** Kaplan-Meier graph of Fc γ RIIIa displaying time to event analysis for progression. Failure was defined as the time to the first of three failure events: CD4 count <200, ART initiation, or death. The survival curves represent the different receptors (*solid line* = FF, *dashed line* = FV, and *dotted line* = VV).

95% CI -0.62 to 4.48 , $p=0.057$), and became significant after adjustment (beta = 2.62 , 95% CI 0.26 to 4.97 , $p=0.029$).

During follow-up, 136 of the 253 (53.8%) women genotyped reached at least one criterion for disease progression (CD4 count <200 cells/mm³, ART initiation, or death). Kaplan-Meier curves were generated by genotype (Fig. 2). Log-rank tests showed no difference in time to disease progression ($p=0.51$ for Fc γ RIIa, $p=0.63$ for Fc γ RIIIa). Modeled with Cox proportional hazards, there was no difference in time to disease progression for women with Fc γ RIIa HR [relative hazard (RH) = $+1.13$, 95% CI 0.63 – 2.01 , $p=0.60$] or RR (RH = $+0.86$, 95% CI 0.44 – 1.66 , $p=0.66$) relative to HH. There was no increased hazard for women with Fc γ RIIIa FV (RH = $+1.01$, 95% CI 0.62 – 1.63 , $p=0.97$) or VV (RH = $+0.80$, 95% CI 0.41 – 1.57 , $p=0.51$) relative to FF. Results for both receptors were unchanged after adjustment or inclusion of an interaction term between genotypes. Because the threshold for ART initiation increased over the study period from a CD4 count of 200 cells/mm³ to 350 cells/mm³, we conducted a sensitivity analysis using the higher cutpoint (350 cells/mm³) to define disease progression. Results were unchanged (data not shown).

We found no association between Fc γ RIIIa nor Fc γ RIIa genotype and set point viral load, viral load change over time, CD4 cell change over time, or overall disease progression. We did detect an association between Fc γ RIIIa VV genotype and a higher CD4 cell count after acute infection (intercept) in the LME model. This association was of borderline significance when unadjusted, but became statistically significant after adjustment for covariates. However, there is no clear biological mechanism that would explain this association. Moreover, the modeled increase in CD4 cells at the VV intercept equates to 6.9 cells/mm³, which is unlikely to have clinical relevance.

Our study does not recapitulate findings in other cohorts, studies that also produced somewhat conflicting results. Forthall *et al.* reported that Fc γ RIIa predicted progression toward CD4 count <200 in a North American cohort of men who have sex with men ($n=550$), with the low-affinity RR homozygotes progressing more rapidly than HR or HH.¹³ They also found that RR homozygotes had less efficient phagocytosis of HIV-1/IgG complexes. In contrast, Poonia *et al.* found no association between the distribution of

Fc γ RIIa genotypes in a group of “normal HIV-1 progressors” ($n=59$) and a cohort of natural virus suppressors ($n=43$), but instead reported an increased risk of disease progression in Fc γ RIIIa high-affinity VV homozygotes relative to low-affinity FF homozygotes.¹⁴ It is unclear if these differences from the present study reflect dissimilarities in the role of Fc γ R receptor affinity in different populations or in women compared to men, or could reflect other methodological differences.

The strength of our study is the use of samples from a cohort of >250 HIV-1 seroconverters with close follow-up and a reasonably precise estimate of infection timing. Many women in this study have been followed for more than a decade. However, we acknowledge several limitations. First, many women had long intervals between visits or were lost to follow-up. Second, CD4 cell counts became a routine part of clinical follow-up only in 1998. This may introduce a calendar year bias, since CD4 counts were not available for use in the CD4 LME and survival analyses before this time. Third, the women in this study represented several Kenyan ethnic groups, and there is likely genetic variation between these groups that is not accounted for in our analyses. Finally, not all potential predictors were tested. For example, Li *et al.* recently identified a polymorphism in Fc γ RIIc that was associated with vaccine protection in the RV144 Thai trial²⁷; in addition, there are other known polymorphisms and copy number variants in Fc γ Rs that could be predictors of disease progression.

This study is the first to examine the roles of Fc γ RIIa and Fc γ RIIIa genetic polymorphisms in HIV-1 disease progression in African women, and focused on women with a known window of infection. Our results suggest that polymorphisms in the Fc γ receptors may be less important to viral control and disease progression in this population, in which other host or viral factors may be more important determinants.

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Author Disclosure Statement

No competing financial interests exist.

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